REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ORGANIZATION.

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
12-09-2016	Interim	01SEP2014 - 30APR2016

4. TITLE AND SUBTITLE

Umbilical Cord Blood Use For Admission Blood Tests of VLBW (very low birth weight) Preterm Neonates: A Multi-center Randomized Clinical Trial

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Alicia Prescott, DO; Jonathan Shapiro, MD; Levi Funches, MD;
Brenda Stryjewski, MD; Thornton Mu, MD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

San Antonio Military Medical Center
3551 Roger Brooke Dr.
Fort Sam Houston, TX 78234

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

SAMMC IRB
3551 Roger Brooke Dr.
Fort Sam Houston, TX 78234

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

A - Public release

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Very low birth weight premature neonates undergo phlebotomy procedures on admission to the neonatal intensive care unit that require 10% of their total blood volume. Increasing their likelihood of anemia requiring packed red blood cell transfusion. A strategy to decrease the risk of anemia includes use of umbilical cord blood for admission lab tests. Our objective is to compare the use of umbilical cord vs. infant blood as a strategy to decrease the risk of anemia requiring PRBC transfusion in VLBW infants. Study was designed as a multicenter, prospective, randomized control trial. Participants are infants less than 30 weeks or birth weight less than 1200 grams. Participants randomized to the experimental arm have admission labs drawn from umbilical cord blood. Labs from the control arm are drawn from the infant. The primary outcomes are absolute hemoglobin concentration and percent change in HgB concentration from baseline to 24 hours of life. Interim analysis of 44 patients was performed. The percent change in HgB concentration from baseline to 24 hours of life in the control arm was -3.54% compared with a 7.46% increased HgB concentration in the experimental arm ($p = 0.018$). There was also a trend toward higher HgB concentration at 24 hours in the

15. SUBJECT TERMS

Anemia, umbilical cord blood, VLBW Infants

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Alicia Prescott, Maj, USAF, MC
UU	UU	UU	SAR	8	19b. TELEPHONE NUMBER *(Include area code)* 858 - 232- 8459

Reset

Umbilical Cord Blood Use for Admission Blood Tests of VLBW Preterm Neonates: Interim Data Analysis from a Multi-Center Randomized Clinical Trial

Alicia Prescott, DO[1]; Jonathan Shapiro, MD[1]; Levi Funches, MD[2];
Brenda Stryjewski, MD[3]; Thornton Mu, MD[1]

[1]San Antonio Military Medical Center [2]Madigan Army Medical Center [3]Tripler Army Medical Center

Introduction

Very low birth weight (VLBW) premature neonates typically undergo phlebotomy procedures at the time of admission to the neonatal intensive care unit (NICU) as part of the admission evaluation [1, 2]. These blood tests include the following: complete blood count with differential and platelet count (CBC), blood culture, blood gas, blood glucose, and type and cross match. Occasionally it can also include the newborn metabolic screen, karyotype testing, and coagulation profile. The volume of blood required for these tests varies between 3-6mL and for the smallest infants, this can represent up to 10% of their total blood volume[3]. These phlebotomy procedures at the time of admission along with subsequent phlebotomy procedures throughout their NICU course, increases an infants likelihood of anemia requiring packed red blood cell (pRBC) transfusions[4, 5].

Strategies exist to help decrease the risk of anemia and the need for transfusion. These include delayed cord clamping, cord stripping, erythropoiesis stimulating agents (erythropoietin and darbepoetin alfa), and limiting phlebotomy via use of point-of-care testing devices, benchtop laboratory analyzers, and transcutaneous measurements[3, 6]. Another approach used to decrease the risk of anemia and need for transfusion is the use of umbilical cord blood, drawn immediately following placental delivery, for NICU admission laboratory tests[7]. Studies have shown that umbilical cord blood drawn immediately following placental delivery is equivalent to blood drawn from neonates for complete blood count parameters and blood culture analyses[8-11]. Furthermore, Baer *et al.* recently reported a case-control study where neonates whose admission lab work was obtained from the umbilical cord blood resulted in higher hemoglobin concentrations, less vasopressor use, and fewer transfusions in the first week of life[12].

To date, there have been no randomized clinical trials to investigate the use of umbilical cord blood for NICU admission blood work in VLBW infants. Our goal was to establish the first randomized control trial comparing the use of cord blood versus infant blood for NICU admission lab tests.

Methods

This study is a multicenter randomized control trial (RCT) across three participating neonatal centers comparing two treatment arms, the use of infant blood for NICU admission lab tests versus the use of cord blood.

Study Population
Participants were infants born less than 30 weeks gestation or whose birthweight was less than 1200 grams born at one of three military treatment facilities (MTFs) that routinely care for VLBW premature neonates in level 3 NICUs. All pregnant women presenting to labor and delivery for possible preterm delivery prior to 30 weeks were approached for consent to enroll their neonates into the study. There were no specific exclusion criteria, however infants transferred out of participating institutions during the first week of life were excluded from analysis.

Randomization
Competitive enrollment occurred across all participating sites utilizing simple randomization at a 1:1 allocation ratio. Randomization schedule was provided via closed envelopes by the 59th Medical Wing statistician for dissemination to each of the participating sites.

Interventions
The control arm received standard of care practice with respect to obtaining admission blood work from the infant at the time of admission into the NICU. The experimental arm had admission labs drawn from the umbilical cord blood. The technique used for obtaining blood from the umbilical cord has been described previously [7, 12]. A complete blood count (CBC) was obtained on both groups at 12-24 hours of life and on the 7th day of life to evaluate hemoglobin concentration. Vasopressor use, need for and total volume of pRBC transfusions, number of donor exposures, were monitored in the first week of life and at the time of discharge in all study participants. Head ultrasounds (HUS) were obtained at 7-10 days of life and at 30 days of life in all participants. The presence and severity of intraventricular hemorrhage (IVH) was interpreted by a pediatric radiologist who was blinded to the infants' randomization.

Study Outcomes
The primary outcome was the absolute hemoglobin concentration and the percent change in hemoglobin concentration from baseline at about 24 hours of life. Secondary outcomes included number of transfusions, number of donor exposures, time to first transfusion, vasopressor use, presence of severe IVH, presence of ROP requiring treatment, and the composite outcome of ROP, BPD, IVH or death.

Sample Size
The protocol calls for a sample size of 225 infants, with 180 infants needed to achieve an 80% power to detect a mean difference in HgB concentration of 1.2g/dL. This sample size accounts for a 20% drop out rate. This protocol is on going and this paper is an interim analysis of the first 44 patients.

Statistical Methods

The primary analytic approach was based on intention to treat. Wilcoxon rank sum test was used for non parametric data. Paired t test and χ^2 test were used on parametric data. An outcome was considered statistically significant if P value was < 0.05

Results

A total of 70 women were admitted at less than 30 weeks gestation to labor and delivery at one of three participating institutions. 67 were approached for consent to enroll their infants in the study upon delivery. Three were missed due to failure of on call provider to notify study personnel or inability for study personnel to get to hospital to consent prior to delivery. 66 women consented to have their infants participate in the study. One infant was not enrolled due to parent refusal. Of the 66 infants initially consented for participation, 44 were enrolled in the study. The other 22 eventually delivered at > 30 weeks gestation and no longer met study criteria. The 44 enrolled infants were randomized to either the control group (n = 20) or the treatment group (n = 24) (Fig 1).

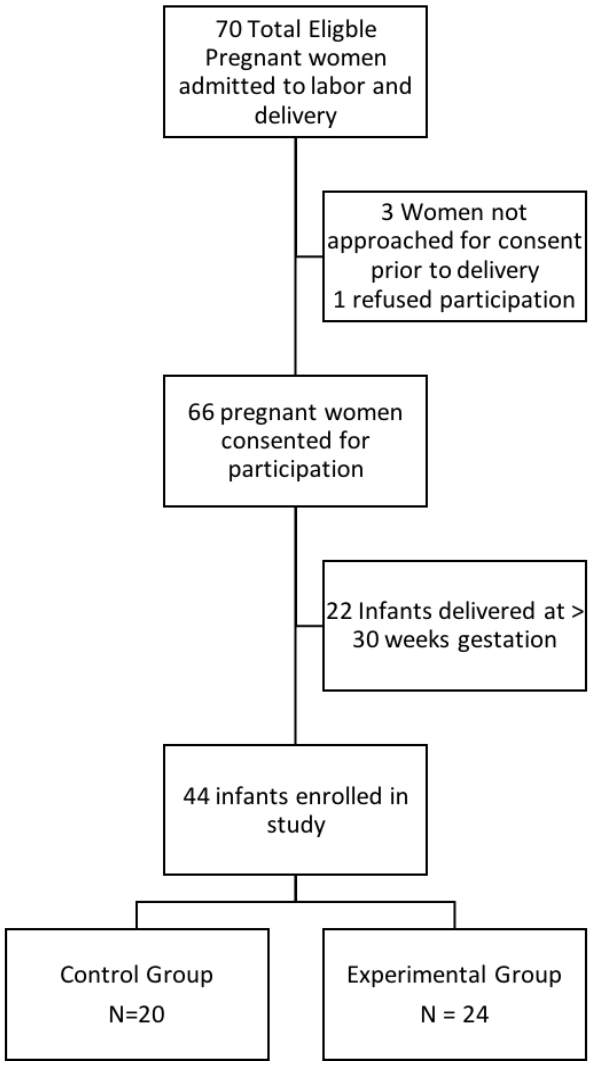

Figure 1: Patient Enrollment

Participant Characteristics

The groups were similar in background characteristics at birth (Table 1). In both groups the birthweight was just over 1000g and infants were birth at around 27-28 weeks. Approximately half were male in both groups with over 60% receiving a full course of antenatal steroids. SNAPPE – II score were similar between groups. APGAR scores were the same for the two groups. There was a slightly higher percentage of delayed cord clamping in the treatment group, however it was not statistically significant.

Table 1: Participant Characteristics

Infant Characteristic	Control Group (N = 20)	Treatment Group (N = 24)
Birthweight (g)	1025.5g $\pm$ 308	1021 $\pm$ 291
Gestational Age (weeks)	27 $\pm$ 3d	28 $\pm$ 3d
Male Gender (%)	9/20 (45)	12/24 (50)
Received antenatal steroids (%)	13/20 (65)	17/24 (62.5)
SNAPPE II Score (n)	29 $\pm$ 22	32 $\pm$ 22
Delayed cord clamping (%)	5/20 (25)	8/24 (33)
Apgar Score 1 minute (n)	4 $\pm$ 2	4 $\pm$ 2
Apgar Score 5 minutes (n)	7 $\pm$ 2	7 $\pm$ 2

Study Outcomes

All study participants had a CBC drawn on admission either from the umbilical cord (treatment group) or the infant. Six of the samples drawn off the cord were not adequate. Five of them were found to be inadequate due to severe thrombocytopenia found on the initial sample that was subsequently proven to be false on repeat sample from the infant. In one infant randomized to the treatment group, not enough blood was obtained from the cord to be able to run a CBC. All samples drawn from the infant were adequate. Of the total of 44 study participants all 44 had a CBC drawn at 12-24 hours. Only 37 of the 44 infants had CBCs obtained on day of life 7. Five were not obtained by the ordering provider at the correct time. Two patients died prior to day of life 7.

There was so significant difference in hemoglobin levels between the treatment and control groups at 12-24 hours of life(14.73 $\pm$ 0.51 vs 13.36 $\pm$ 0.56, P = 0.076) or on day of life 7 (12.18 $\pm$ 0.29 vs 12.42 $\pm$ 0.39, p = 0.611) (Table 2). There was statistically significant percent change in [HgB] from baseline at 12-24 hours of life in the treatment group as compared to the control group (7.02 $\pm$ 3.27 vs -5.13 $\pm$ 3.27 P = 0.006). The control group had an increase in [HgB] from baseline versus the control group that had a drop in [HgB] from baseline at 12-24 hours of life. At seven days of life the % change from baseline is similar in both groups (-8.93 $\pm$ 3.35 vs -8.96 $\pm$ 2.90, p = 0.995).

Most of the secondary outcomes were not statistically significant (Table 4). There was no difference in the number or volume of PRBC transfusions at DOL 7 or at the time of discharge.

At DOL 7 the number of transfusions was 1.14 ± 0.47 in the treatment group vs 1.95 ± 0.54 in the control group (p = 0.259). The volume of PRBCs (mL) at DOL 7 was 12.55 ± 5.18 vs 20.775 ± 5.38 (P = 0.279). There was also no difference in the time to first transfusions (10.14d ± 2.46d vs 8.36d ± 3.30d, p = 0.668). Vasopressor use at DOL 7 was also similar in the two groups (23% vs 35%, p = 0.591).

Table 2: Absolute HgB Concentration (g/dL) on Admission, at 12-24 hours of life, and on day of life 7

	Treatment	Control	P Value
Admission	13.87 ± 0.39	14.08 ± 0.44	0.726
12-24 hours	14.73 ± 0.51	13.36 ± 0.56	0.076
DOL 7	12.18 ± 0.29	12.42 ± 0.39	0.611

Table 3: % change in [HgB] from baseline

	Treatment	Control	P Value
12-24 hours	7.02 ± 3.27	-5.13 ± 3.27	0.006
DOL 7	-8.93 ± 3.35	-8.96 ± 2.90	0.995

The results were similar at the time of discharge. The treatment group had received 4.17 ± 1.27 transfusions vs 6.26 ± 1.48 in the control group (p = 0.289). The total volume (mL) of PRBCs was 41.21 ± 9.03 in the treatment group vs 70.47 ± 15.3 in the control group (p = 0.11). There was no difference in the amount of grade III or IV IVH (12.5% vs 30%, p = 0.29) or ROP requiring treatment (8.3% vs 15%, P = 0.828). However, the composite outcome of ROP requiring treatment, BPD, grade III or IV IVH or Death was significant with at least one of those outcomes occurring in 30.2% of the treatment group versus 69.8% of the control group (p = 0.0006).

Table 4: Secondary Outcomes

Secondary Outcome	Treatment	Control	P Value
Transfusions at DOL 7 (n)	1.14 ± 0.47	1.95 ± 0.54	0.259
Volume of transfusions at DOL 7 (mL)	12.55 ± 5.18	20.75 ± 5.38	0.279
Time to first transfusion (Days)	10.14 ± 2.46	8.36 ± 3.30	0.668
Vasopressor use at DOL 7 (%)	23	35	0.591
Transfusions at discharge (n)	4.17 ± 1.27	6.26 ± 1.48	0.289
Volume of transfusions at discharge (mL)	41.21 ± 9.03	70.47 ± 15.3	0.11
Grade III or IV IVH at discharge (%)	12.5	30	0.29
ROP requiring treatment (%)	8.3	15	0.828
Composite outcome of ROP, BPD, grade III or IV IVH or Death (%)	30.2	69.8	0.0006

Discussion

Packed red blood cell transfusions can provide clinical benefits to infants admitted to the neonatal intensive care unit, but they are not without risks. Risks include transfusion related gut injury, transfusion related lung injury, and increased risk for IVH [13]. It is important to find ways to safely decrease the number of transfusions our neonates receive. Some commonly used nonpharmacologic techniques for decreasing transfusion rates include delayed cord clamping, cord stripping, limiting phlebotomy procedures, and obtaining NICU admission lab tests from umbilical cord blood [3]. In the placenta the maternal and fetal circulation are separated by the trophoblastic membrane so that blood obtained from the umbilical cord vessels is fetal, not maternal [3]. Previous studies have shown that CBC indices, manual differential, and blood culture drawn from this umbilical cord blood can be used reliably [8, 9, 11].

Previous case control studies have been done in premature infants looking at drawing admission labs from the umbilical cord blood versus infant blood, proving the method to be feasible [7, 12]. However, this is the first randomized control trial comparing the use of umbilical cord blood versus infant blood for NICU admission lab tests. Although our study did not look specifically at feasibility, for the first 24 patients randomized to the treatment arm, successful results were obtained from the cord blood 75% of the time. The first few blood draws resulted in a thrombocytopenia that was proven to be false on repeat infant sample. This finding was described by Carroll who concluded that adopting a strategy of using cord blood for admission labs would result in a clinically important number of false-positive cases of thrombocytopenia [9]. We found that by modifying our technique and drawing blood from higher up on the cord, further from the cord's insertion to the placenta, resulted in more reliable platelet counts. More research is needed to understand what factors within the placenta may lead to falsely lowered platelet counts.

We chose to use [HgB] at 12-24 hours, as well as % change from baseline [HgB] at 12-24 hours as our primary outcomes. These outcomes are similar to what was used in previous studies done by both Christensen and Baer [7, 12]. Our interim analysis did not find a statistical difference in the absolute [HgB] at 12-24 hours, however, it did find a significant difference in the % change from baseline [HgB] at 12-24 hours. The treatment group had an increase in [HgB] from baseline, whereas the control group had a decrease in [HgB] at 12-24 hours of life. This confirms what both Baer and Christensen reported in their case control studies. Of note, at the time of the interim analysis, although not statistically significant our treatment group did have a higher proportion of infants who received delayed cord clamping at the time of delivery. Delayed cord clamping has been proven to increase HgB concentration and could be one explanation for this increase in [HgB]. However, Baer showed that in those that had their admission labs drawn off of the cord had higher [HgB] at 12-24 hours of life, when compared to their matched controls, even when they did not receive delayed cord clamping or cord stripping [12]. This supports our conclusion that the increase in [HgB] cannot just be attributed to delayed cord clamping alone.

In both previous case control studies, the authors found a decrease in the number of transfusions during the first week of life as compared to the controls [7, 12]. We did see a trend toward a decrease in the number of transfusions, however, given the limited number of infants in our interim analysis, no statistical significance was seen.

Other secondary outcomes included vasopressor use and rate of severe IVH. Previous studies have reported a statistically significant decrease in vasopressor use in the first week of life, but have had conflicting results in regards to the rate of IVH. Although not an intended outcome, Christensen does report a decrease in the rate of IVH among cases when compared to controls [7]. Baer found no difference in the rate of IVH. We saw a trend towards a decrease in the amount of IVH, however as is the case with most of our secondary outcomes, it is difficult to draw any conclusions as we are not powered correctly. The one secondary outcome that was statistically significant was the composite out come of ROP, BPD, IVH, or death. This outcome was not looked at in previous studies but could have significant implications if it remains significant once enrollment is complete.

Our study is still in its early stages and we remain open to enrollment currently at four sites, with IRB approval pending at a fifth site. Given that fact, our study still has several strengths. It is a prospective, multicenter, randomized control trial and ultimately, our enrollment goal would make it the largest population studied to date. We chose [HgB] concentration as a primary outcome versus a more clinically significant primary outcome such as number of pRBC transfusions, as it would take a much larger study to be powered in order to detect a difference in the number of transfusions. We believe that absolute [HgB] is a translates well to clinically significant outcomes. Weaknesses include the current limited enrollment and the inability to blind randomization to the providers caring for the infant.

It is difficult to draw definitive conclusions off the small number of patients included in our interim analysis. However, preliminary data suggest that the use of cord blood for admission labs result in higher percentage of HgB concentration in the first 24 hours of life at a period of higher risk for hemodynamic instability.

References

1. Bishara, N. and R.K. Ohls, *Current controversies in the management of the anemia of prematurity.* Semin Perinatol, 2009. **33**(1): p. 29-34.
2. Lin, J.C., et al., *Phlebotomy overdraw in the neonatal intensive care nursery.* Pediatrics, 2000. **106**(2): p. E19.
3. Carroll, P.D. and J.A. Widness, *Nonpharmacological, blood conservation techniques for preventing neonatal anemia--effective and promising strategies for reducing transfusion.* Semin Perinatol, 2012. **36**(4): p. 232-43.
4. Jansen, M., et al., *Potential use of autologous umbilical cord blood red blood cells for early transfusion needs of premature infants.* Transfusion, 2006. **46**(6): p. 1049-56.
5. Meyer, E.K. and C.D. Josephson, *Neonatal and pediatric transfusion practice.* American Acadamey of Blood Banking Technical Manual, 2014. **18**: p. 571-592.

6. Ohls, R.K., et al., *A randomized, masked, placebo-controlled study of darbepoetin alfa in preterm infants.* Pediatrics, 2013. **132**(1): p. e119-27.
7. Christensen, R.D., et al., *Postponing or eliminating red blood cell transfusions of very low birth weight neonates by obtaining all baseline laboratory blood tests from otherwise discarded fetal blood in the placenta.* Transfusion, 2011. **51**(2): p. 253-8.
8. Beeram, M.R., et al., *Utilization of umbilical cord blood for the evaluation of group B streptococcal sepsis screening.* Clin Pediatr (Phila), 2012. **51**(5): p. 447-53.
9. Carroll, P.D., et al., *Umbilical cord blood as a replacement source for admission complete blood count in premature infants.* J Perinatol, 2012. **32**(2): p. 97-102.
10. Costakos, D.T., et al., *Painless blood testing to prevent neonatal sepsis.* Wmj, 2009. **108**(6): p. 321-2.
11. Hansen, A., P. Forbes, and R. Buck, *Potential substitution of cord blood for infant blood in the neonatal sepsis evaluation.* Biol Neonate, 2005. **88**(1): p. 12-8.
12. Baer, V.L., et al., *Using umbilical cord blood for the initial blood tests of VLBW neonates results in higher hemoglobin and fewer RBC transfusions.* J Perinatol, 2013. **33**(5): p. 363-5.
13. Christensen, R.D. and S. Ilstrup, *Recent advances toward defining the benefits and risks of erythrocyte transfusions in neonates.* Arch Dis Child Fetal Neonatal Ed, 2013. **98**(4): p. F365-72.